In this regard, the specification has been amended so that it is commensurate with the submission of the present Sequence Listing. In light of the enclosed Sequence Listing, Figures 3.1 to 3.9 are superfluous. As such, applicants respectfully ask that Figures 3.1 to 3.9 be canceled and that Figures 4.1, 4.2, 4.3, 5, 6a and 6b be renumbered 3.1, 3.2, 3.3, 4, 5a and 5b respectively. Enclosed with this response are the proposed changes to the figures in red ink.

A6

In view of the above, it is respectfully submitted that the above-identified application complies with the requirements for patent applications containing nucleotide sequence and/or amino sequence disclosures.

Favorable consideration of this application is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

YOUNG & THOMPSON

By Philip A. DuBois

Philip A. DuBois
Agent for Applicants
Registration No. 50,696
745 South 23rd Street
Arlington, VA 22202
Telephone: 521-2297

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at line 10 of page 3 has been amended as follows:

The nucleotide and amino acid sequence of the novel glucosyltransferase are represented by SEQ ID No. 1 and 2, respectively[, and are also shown in figure 3].

Paragraph beginning at line 12 of page 3 has been amended as follows:

The start codon of the glucosyltransferase is preceded by a putative ribosome binding site with the nucleotide sequence GAAGGAGA (located 5 base pairs upstream of the start codon of the glucosyltransferase; see SEQ ID No. 1 [and figure 3]). Furthermore, the start codon is preceded by the nucleotide sequence TATAAT, also called Pribnow box or -10 region, (located 42 base pairs upstream of the start codon; see SEQ ID No. 1 [and figure 3]) and by the nucleotide sequence TTGAAA, also called -35 region (located 80 base pairs upstream of the start codon; see SEQ ID No. 1 [and figure 3]).

Paragraph beginning at line 20 of page 3 has been amended as follows:

The invention covers a protein having glucosyltransferase activity with sucrose as substrate comprising an amino acid sequence with an amino acid identity of at least 50%, preferably at least 60%, and more preferably at least 70%,

compared to the amino acid sequence 531-1781 of SEQ ID No. 2. The invention also covers a part of a protein with at least 15 contiguous amino acids which are identical to the corresponding part of the amino acid sequence 531-1781 of SEQ ID No. 2. The present invention covers a protein having glucosyltransferase activity with sucrose as substrate with an amino acid identity of at least 50%, preferably at least 60%, and more preferably at least 70%, compared to the amino acid sequence of SEQ ID No. 2. The invention also covers a part of a protein with at least 15 contiguous amino acids which are identical to the corresponding part of the amino acid sequence of SEQ ID No. 2. The novel glucosyltransferase has homology with several other proteins as revealed by amino acid sequence alignment. A high homology (figure [5] 4) was found with an alternansucrase of *Leuconostoc mesenteroides* strain NRRL B-1355 (46% identity, within 1261 amino acids of amino acid sequence 531-1781 of SEQ ID No. 2) and a dextransucrase of *Leuconostoc mesenteroides* strain NRRL B-512F (44% identity, within 1270 amino acids of amino acid sequence 531-1781 of SEQ ID No. 2). Furthermore, the alignment revealed the presence of various domains also found in the other glycosyltransferases, such as an N-terminal variable domain, a catalytic domain and a C-terminal glucan binding domain. The N-terminal domain shows almost no identity with the N-terminal domains of other glucosyltransferases and an N-terminal signal peptide could not be detected.

Paragraph beginning at line 8 of page 4 has been amended as follows:

The invention also covers a protein comprising an amino acid sequence of at least 100 amino acids, exhibiting at least 55%, preferably at least 65% amino acid identity with the corresponding part of the amino acid sequence 972-1514 (catalytic domain) of SEQ ID No. 2. The catalytic domain shows a high level of homology (about 50% identity) with other known streptococcal and *Leuconostoc* glucosyltransferases and putative functions based on the alignment can be ascribed to several amino acids within this catalytic domain (figure [4] 3). Asp-1024, Glu-1061 and Asp-1133 of SEQ ID No. 2 are putative catalytic residues, Asp-984 of SEQ ID No. 2 is a putative calcium binding residue and Arg-1022 of SEQ ID No. 2 a putative chloride binding residue. His-1132 and Gln-1514 of SEQ ID No. 2 may stabilize the transition state and the residues Asp-1027, Asn-1028, Asp-1062 and Trp-1063 of SEQ ID No. 2 may play a role in binding of acceptor molecules and in the transfer of the glucosyl moiety.

Paragraph beginning at line 16 of page 5 has been amended as follows:

A nucleotide sequence encoding any of the above mentioned proteins, mutants, variants or parts thereof is also a subject of the invention. Furthermore, the nucleic acid sequences corresponding to expression-regulating regions (promoters, enhancers, terminators) contained in the nucleic acid

sequence (1)-(160) or (5507)-(6026) of SEQ ID No. 1 [(see also figure 3)] can be used for homologous or heterologous expression of genes. Such expression-regulating sequences are operationally linked to a polypeptide-encoding nucleic acid sequence such as the genes of the glucosyltransferase according to the invention. Inverted repeats are located 62 base pairs downstream the termination codon (TAA), suggesting the presence of a Rho independent transcription termination signal. The -10 and -35 consensus promoter sequences, two motifs generally present upstream of the start codon of procaryotes, are identified as described above. Other promoter, enhancer or terminator were not identified. A nucleic acid construct comprising the nucleotide sequence operationally linked to an expression-regulating nucleic acid sequence is also covered by the invention.

Paragraph beginning at line 19 of page 13 has been amended as follows:

The glucosyltransferase activity was determined at 37°C by monitoring the release of fructose from sucrose or by measuring the amount of clucan produced using *E. coli* cell free extracts or *Lactobacillus reuteri* culture supernatant in reaction buffer (50 mM sodium acetate, 1 mM $CaCl_2$, 1% (v/v) Tween-80, 10 g/l sucrose, pH 8). Sucrose, glucose and fructose were determined using commercially available kits. For determination of the molecular weight of the glucosyltransferase produced by *E. coli* or *Lactobacillus reuteri*, SDS-PAGE was performed according

to Laemmli (1970) Nature 227, 680-685. SDS-PAGE gels were stained using the PAS activity staining. Glucans were collected by precipitation with ethanol. ^{1}H-NMR spectroscopy (figure [6] 5) and methylation analysis (table 1) were performed as described by van Geel-Schutten *et al.* (1999) Appl. Environ. Microbiol. 65, 3008-3014. The molecular weights of the glucans were determined by high performance size exclusion chromotography coupled on-line with a multi angle laser light scattering and a differential refractive index detector. After the first nucleotide sequencing of the obtained DNA two putative start codons leading to either a protein encoded by 3834 nucleotides (starting at nucleotide position 1670 of SEQ ID No. 1) or a protein encoded by 3753 nucleotides (starting at nucleotide position 1751 of SEQ ID No. 1) were identified. Both putative start codons were preceded by a putative ribosome binding site, GCAGG (located 4 base pairs upstream of nucleotide position 1751 of SEQ ID No. 1), respectively. At the beginning it was believed that the above mentioned nucleotide forms encoded two glucosyltransferases. Depending on the potential start codon used, one of these glucosyltransferases comprised 1278 amino acids (starting at amino acid positions 504 of SEQ ID No. 2) (3834 nucleotides) and the other comprised 1251 amino acids (starting at amino acid position 531 of SEQ ID No. 2) (3753 nucleotides). The molecular weight (MW) deduced of the amino acid sequence of these glycosyltransferases was 143 and 140 kDa, respectively. The

isoelectric point deduced of the amino acid sequence of these glucosyltransferases was 4.73 (for the higher MW protein) and 4.71 (for the lower MW protein), at pH 7, respectively. Surprisingly, the molecular weight of the purified protein from *Lactobacillus reuteri* indicated by SDS-PAGE was not approximately 140 kDa but 180 kDa. After repeating the nucleotide sequencing, it appeared that the above mentioned nucleotide forms did not represent the complete nucleotide sequence of the glucosyltransferase according to the invention, but was merely a part of the complete nucleotide sequence encoding the protein of the invention. The complete nucleotide sequence of the novel glucosyltransferase is represented in SEQ ID No. 1 and the amino acid sequence of said glucosyltransferase is shown in SEQ ID No. 2. All experiments were performed with both the complete nucleotide or amino acid sequence of the protein (SEQ ID No. 1 and 2, respectively) and the partial nucleotide or amino acid sequence mentioned above. The results of the experiments performed with the complete or partial amino acid sequence and the complete or partial nucleotide sequence mentioned above were identical indicating that the part of the glucosyltransferase represented by said partial nucleotide and amino acid sequences is essential for the functionality of the glucosyltransferase according to the invention.

Paragraph beginning at line 30 of page 18 and ending at line 2 of page 19 has been cancelled.

Paragraph beginning at line 3 of page 19 has been amended as follows:

Figure [4] 3: Alignment of catalytic cores of alternansucrase (ASR) of *Leuconostoc mesenteroides* strain NRRL B-1355 dextransucrase (DSRS) of *Leuconostoc mesenteroides* strain NRRL B-512F, glucosyltransferase-D (GTFD) of *Streptococcus mutans* GS5, glucosyltransferase-A of *Lactobacillus reuteri* and amylosucrase (AS) of *Neisseria polysaccharea*. * indicates identical or conserved residues in all sequences); --- , gap in the sequence; **AA** amino acids which are conserved in all other glucosyltransferases but not in GTFA; ↓, putative catalytic residues; •, putative calcium binding sites; ♦, putative residues stabilizing the transition state; ∇, residues possibly playing a role in binding of acceptor molecules and in the transfer of the glucosyl residue; ◊, putative chloride binding sites; **-Ex-**, localization of β-strands; **-Hx-**, localization of α-helices according to Mac Gregor *et al.* (1996) FEBS Lett. 378, 262-266. The numbering of the amino acids of the glucosyltransferase-A of *Lactobacillus reuteri* corresponds to the positions of these amino acids in the amino acid sequence 531-1781 of amino acid sequence SEQ ID No. 2, when the amino acid sequence 531-1781 is renumbered 1-1251. In figures 3.1 - 3.3, GTFD corresponds to SEQ ID NO. 10. DSRS corresponds to SEQ ID No. 11. ASR corresponds to SEQ ID NO. 12. GTFA corresponds to SEQ ID NO. 13. $QWDLN_2$ corresponds to SEQID NO. 14. IURMDAVAFI corresponds to SEQ ID NO. 15. FVRS

corresponds to SEQ ID NO. 16. GLPRIYLGD corresponds to SEQ ID NO. 17. GLLTYLHLMP corresponds to SEQ ID NO. 18. DFITNH corresponds to SEQ ID NO. 19.